## 31. Experiments on the Metamorphosis of the Mexican Axolotl (*Amblystoma tigrinum*), conducted in the Society's Gardens. By E. G. Boulenger, F.Z.S., Curator of Reptiles.

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(Text-figures 75 & 76.)

During the past year I have been experimenting on points relating to the metamorphosis of the Mexican Axolotl (*Amblystoma tigrinum*), and have succeeded in obtaining the transformation of a number of specimens. This paper deals with the methods employed, and gives a detailed account of the external changes undergone by the animal during the process, a subject which, with the exception of Duméril's (1) brief account, has not, so far as I am aware, been previously treated.

Before describing my experiments, it may not be out of place to give a short account of the history of the animal. Although suspected by Cuvier to be but the larva of some unknown air-breathing salamander or newt, the Axolotl of Mexico was considered for many years to be one of the Perennibranchiata, such as *Proteus*, *Necturus*, and *Siren*. In the year 1863 a number of specimens were imported from Mexico to France, where some, kept in the Jardin des Plantes in Paris, bred, and the young were successfully reared. The conclusion was not unnaturally drawn, that the Axolotl, having bred in the branchiate condition, could not possibly be anything but a perfect aquatic animal. It was, not, however, until over two years later that the subject assumed a different aspect, for some individuals of the second generation lost their gills and the dermal folds of the back and tail, developed eyelids, and yellow spots on the skin, and taking to land, changed into a land salamander, already well known from North America as *Amblystoma tigrinum*.

A few years later Weismann (2) tried to solve the question as to whether it were possible to force the larvæ, if brought into conditions which rendered the use of the gills difficult and that of the lungs easy, to change into Amblystomes, and he therefore experimented with several broods which were placed in shallow water, and thus compelled to breathe air more frequently. Although he met with no success Weismann was not discouraged, and came to the conclusion that the failure of his experiments was due to his having been unable to bestow the necessary care and attention on the animals: he therefore asked Mlle. de Chauvin, a lady who had already done much careful experimental work, to take a number of larvæ, just out of the egg, rear them, and make an attempt to bring them to the perfect condition. When the Axolotls were about 6 months old, Mlle. de Chauvin placed

them in large glass vessels which were so disposed and the water so restricted that at one spot only could they dive quite under, while everywhere else they came into contact with the air. The water was then gradually reduced. Within a few days a change took place, the creatures leaving the water in from four to fourteen days, the complete metamorphosis following about ten days later. Mlle. de Chauvin (3) summarized her results as follows :—" From what I have said, the correctness of the view suggested by Weismann must be established, namely that most Axolotl larvæ, if not all, complete their metamorphosis, if in the first place they come out of the egg healthy and are properly fed, and in the second place meet with arrangements which force them to change from breathing under water to breathing above water."

Dr. J. H. Powers (4) at Doane College, Nebraska, has more recently conducted numerous experiments on the metamorphosis of North American examples of the Axolotl, and he has come to the conclusion that the metamorphosis is not due, as was thought by Mlle. de Chauvin, to a direct response to changes in conditions of environment, compelling them to resort to aerial respiration, but to checked nutrition, and that a careful study of Mlle. de Chauvin's methods and results seems to cast a doubt upon the conclusion that enforced air-breathing caused the metamorphosis. The following is a passage from Dr. Powers's paper on the subject of this lady's experiments :—" Fearing that her charges would die, as indeed they sometimes did, she always prepared them for the trying ordeal of metamorphosis by raising the temperature of the water in which they were kept and feeding to the maximum for several days, to which she ascribes no other importance than giving the animals increased strength. The Axolotls were then brought immediately into water sufficiently shallow as to force them, at least part of the time, to breathe air. In this latter condition the experimenter complains again and again that it was next to impossible to induce the Axolotls to take any food whatever. Thus in these experiments we have high feeding followed by practical starvation, and it seems that no control experiments were instituted to determine what the effects of over and under nutrition might have been with Axolotls still in abundance of water. Yet most interesting is it to note that even the varying factors of nutrition seem to have been wholly neglected in the final interpretation of the results."

Dr. Powers's field-notes show that metamorphosis occurs rarely, if ever, as the result of enforced air-breathing through the drying up of ponds, and that in spite of repeated search at appropriate times and places, no Axolotls have been found transforming on the mud of drying ponds.

Dr. Gadow (5), who not long ago visited the lakes near Mexico City in which this creature lives, and where it is said to retain its branchiate condition, has been able to refute the theories framed

by various zoologists, as to why the Axolotl does not transform in those localities. The reason he gives is that the unfailing abundance of food and water, and the innumerable hiding-places amongst the reeds under the banks, constitute for these Batrachians a real paradise where they remain, in spite of the fact that there is nothing to prevent them from leaving the water. Dr. Gadow's explanation appears to be in keeping with the results of both Mlle. de Chauvin's and Dr. Powers's experiments.

The conflicting conclusions arrived at made it highly desirable that further experiments should be undertaken. The lack of success obtained by the majority of those who have attempted to force the Axolotl to transform, has led many zoologists to believe that the change is due to some congenital disposition, possessed only by certain individuals, and that Mlle. de Chauvin and the few others who have succeeded in obtaining Amblystomes from the larval form were specially fortunate in the choice of their subjects. Therefore, when I commenced my experiments, I was by no means sanguine of attaining any definite results.

In August of last year I obtained five Axolotls with very fully developed gills and fins, ranging in length from 105 mm. to 138 mm., and therefore probably from six to nine months old.

The enforced breathing of air, by the gradual absorption of the water, and by the gradual elimination of the necessary amount of oxygen from the water, starvation and irregular feeding, and increased temperature having all been given or suggested as means of obtaining the metamorphosis, I placed the animals under the following conditions:—

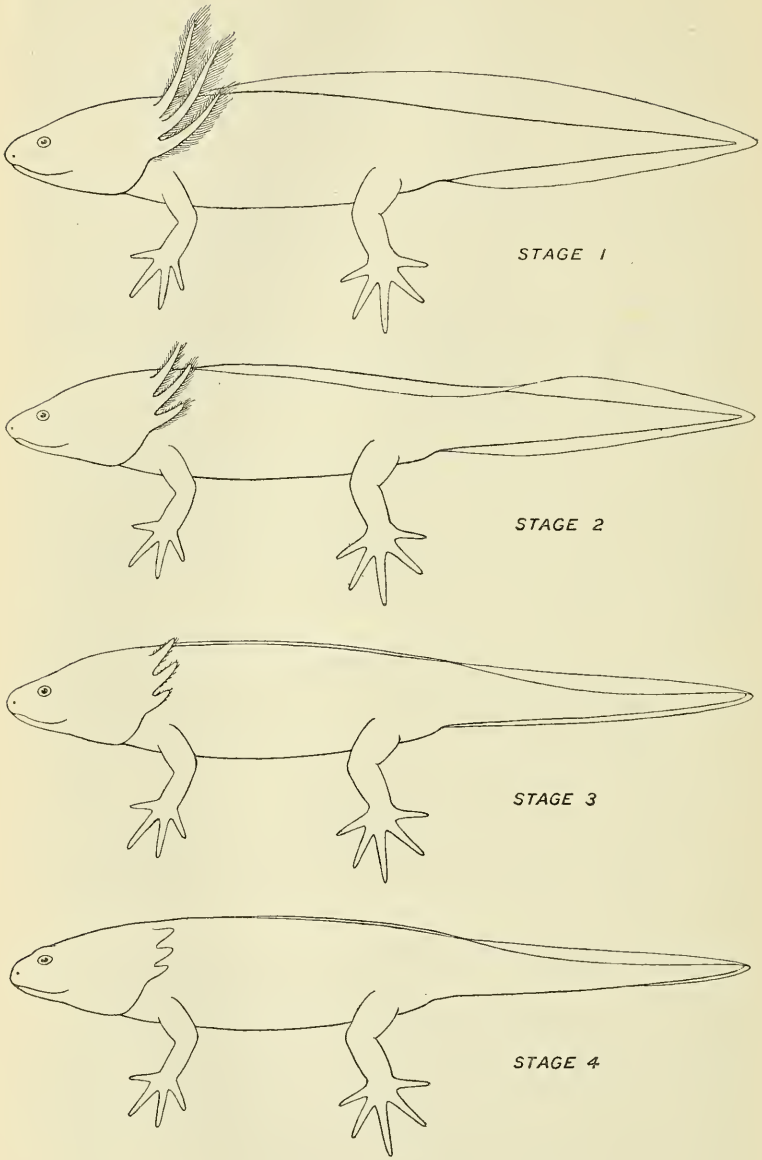
Nos. 1 & 2.—In shallow water so that after the first week only the top of the animal's head and fins remained uncovered, thus forcing the creature to make free use of its lungs. These I kept at a uniform temperature of between 75° and 80°.

Nos. 3 & 4.—Under similar conditions, but at a uniform temperature of between 55° and 60°.

No. 5.—In deep water from which the necessary amount of oxygen was eliminated by the gradual substitution of boiled water and by the introduction of decomposing vegetable matter.

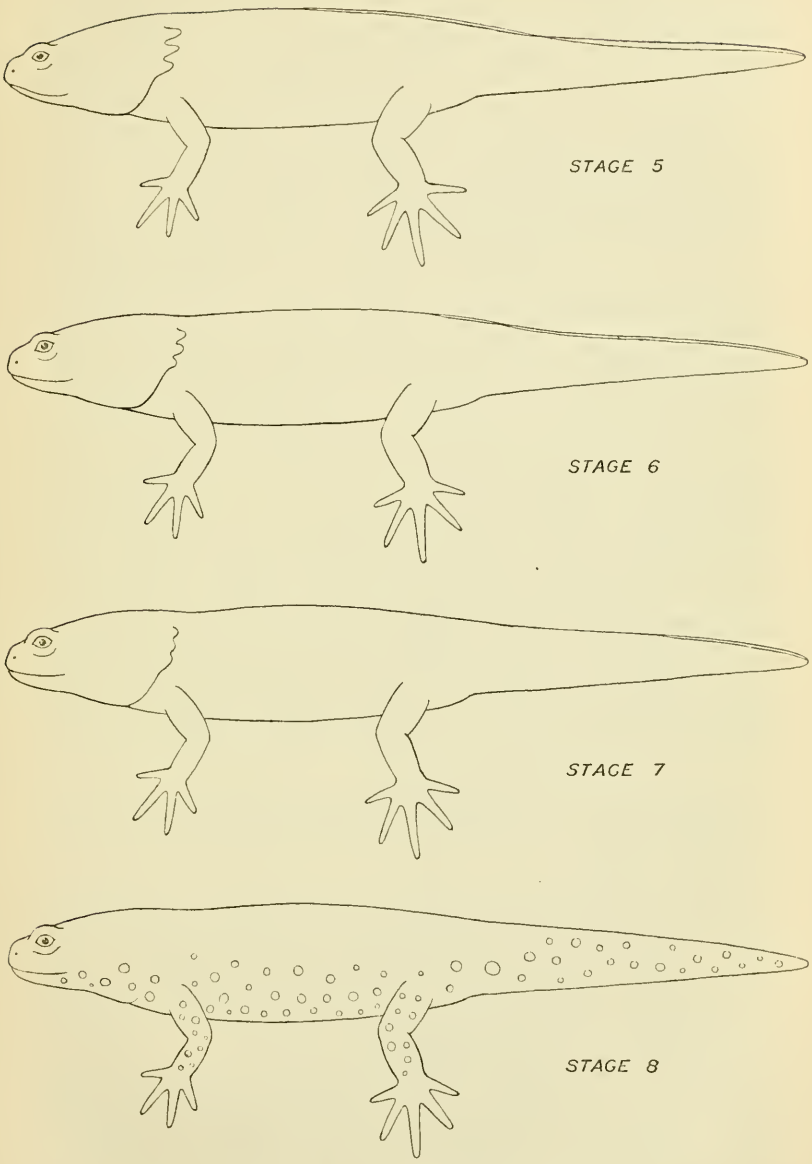
As at the time I could not obtain any more Axolotls of the size required, I decided to abstain from experimenting with the feeding, until I had either obtained negative results with those I was about to experiment on or had secured more suitable specimens. Nearly a month later I was fortunate enough to obtain six further suitable specimens. At the same time some of the first brood showed signs of metamorphosing in spite of the fact that they had fed with the greatest regularity. I therefore decided not to abstain from offering them food as success seemed likely without resorting to starvation, and placed the individuals of the second brood, which shall be referred to as numbers 6, 7, 8, 9,

Text-fig. 75.




First stages in the metamorphosis of *Amblystoma tigrinum*.

Text-fig. 76.




Final stages in the metamorphosis of *Ambystoma tigrinum*.

10, and 11, under similar conditions to those of brood 1. Nos. 6 and 7 were forced to breathe air, being placed in shallow water, at a high temperature; Nos. 8, 9, and 10 were placed under the same conditions at a normal temperature, and No. 11 was placed along with No. 5 in foul water, from which the oxygen was removed by the addition of boiled water. In all, therefore, I had eleven specimens under artificial conditions with the object of forcing the metamorphosis, and as a result succeeded in bringing six individuals to transform into the Amblystome stage, all six specimens being those induced to make free use of their lungs by being placed in shallow water; three of these were kept at a temperature of between 75° and 80°, three at between 55° and 60°. The time required for the entire metamorphosis was from 12 to 16 weeks, periods which all to a remarkable degree exceeded those taken by Mlle. de Chauvin's specimens. Three individuals kept under identical conditions died in from $3\frac{1}{2}$ months to over 5 months, but not until after having undergone a change, which, although considerable, did not necessarily suggest, as will be referred to later, that, but for their death, the entire metamorphosis would have taken place. The two specimens kept in deep water from which the oxygen was eliminated died in the one case after 19 weeks, in the other after 22 weeks. These specimens, but for a slight reduction in the size of their gills, did not undergo any change, the fins being at the time of their death as well developed as at the commencement of the experiment. It is worthy of note that these specimens, in spite of the fact that they must have felt exceedingly uncomfortable, did not rise to the surface for air, even just prior to their death, more frequently than individuals kept in oxygenated water. Another point of interest lies in the fact that both specimens grew rapidly during this period, No. 5 from 136 mm. to 155 mm., No. 11 from 112 mm. to 120 mm. The specimens kept in shallow water, in the case of those that died, remained stationary, while those that transformed actually decreased in size.

Text-figures 75 and 76 (pp. 406 and 407) represent eight stages through which my Axolotls passed in the course of their metamorphosis.

Stage 1 represents the perfect larval form, the condition which all eleven were in before being placed in shallow water. A few days after the animals had been placed in shallow water, the gills with their fringes began to shrink, and the dorso-caudal fin which lopped over to one side, to the left side in all my specimens, became reduced in size, the change being plainly visible in from a fortnight to three weeks (Stage 2).

In from 10 to 20 days later the gills were only half their normal size, while the fin of the back was represented by a mere ridge; that of the tail, although having undergone further reduction, was still well developed on the upper surface and flopped over to one side; on the lower surface, however, it showed considerable reduction (Stage 3). It was not, however, until from five to seven weeks later (Stage 4) that the metamorphosis proper

took place, when the part of the dorsal fin nearest to the head began to be entirely absorbed; at the same time the head became shorter, assuming a more Salamander-like shape, while a swelling above the eye foreshadowed the advent of the upper eyelid; the gills at this stage measured but from 3 to 5 mm.; the fin of the upper edge of the tail, although now much reduced, still lopped over to one side; the lower fin, but for a rudiment at the extreme end, had entirely disappeared.

Stage 5 represents the conditions seven to eight days later, when the dorsal fin had retreated to the middle of the back; the caudal fin on the lower surface had entirely disappeared; the head had quite altered in aspect, having become much shorter, giving the eyes the impression of having been shifted forwards. At this stage the upper eyelid had become completely formed, the lower eyelid being incomplete.

In from seven to nine days later (Stage 6), the fin of the back had receded to a level with the hind limbs, while on the upper surface of the tail, where it still lopped over, it measured only 1 mm. Both eyelids were now formed. At this stage the animals raised their heads completely out of the water, and the opportunities they were given of getting on land were taken advantage of. Stage 7 represents the creatures about a week later, when nothing remained of the fin but a mere rudiment bordering the extreme end of the upper surface of the tail. The gills were represented by three knobs. Stage 8, the perfect form, the gill slits having closed up and the tail having become more or less roundish, was attained five to eight days later. In the case of both Duméril's and Mlle. de Chauvin's specimens the yellow spots appeared several days prior to the complete metamorphosis; in all my specimens, however, it was not until after they had attained the perfect Amblystome condition that the yellow spots put in an appearance, these being situated on the sides of the body, tail, limbs, and throat; none appeared on the back.

As I was desirous of ascertaining whether the metamorphosis could be checked and the shrinking gills and fins made to undergo fresh development, and if so, at what stage it could no longer be checked, two specimens were taken out of the shallow water and replaced in deep water as they reached successive stages in their development. No. 6 was kept at a temperature of between 75° and 80°, and No. 9 at a temperature of between 55° and 60°. Both these Axolotls were placed in shallow water and then induced to breathe air frequently by means of their lungs on October 7th. Stage 2 was reached on October 20th; they were then replaced in deep water. The gills grew immediately and the fins developed, Stage 1 being reached again on October 28th and November 5th. They were then once more put into shallow vessels, with the result that the gills and fins again began to shrink, Stage 2 being attained on November 15th and 20th, Stage 3 on December 5th and 26th. On the latter dates they were again transferred to deep water, Stage 2 being attained on December 20th and January 10th. The metamorphosis was continued on placing the

Axolotls in shallow water, Stage 3 being reached on January 10th and 25th, and Stage 4, the stage when the front part of the fin becomes totally absorbed, on February 12th and 28th. The Axolotls were then returned to deep water: the development at this stage, however, could no longer be checked and both creatures completed their metamorphosis in the water.

From the latter experiment, showing that when replaced in deep water the previous stages were resumed in about half the time required to reach them, the conclusion may I think be drawn that the natural tendency undoubtedly is for the animal to remain an Axolotl, and that compulsion is needed to bring about metamorphosis.

It will be noted that at Stage 4, when the front part of the fin becomes absorbed, the metamorphosis can no longer be checked, and that this is therefore to be regarded as the critical stage, at which the further development must proceed whatever the conditions under which the animal is placed.

During the course of all my experiments, with the exception of the last stages of the metamorphosis, the Axolotls fed with regularity twice a week, and in this respect I experienced none of Mlle. de Chauvin's difficulties.

Below will be found detailed accounts of the conditions under which my eleven specimens were kept, and of the changes which took place under the circumstances.

*Specimen No. 1.*

Placed in shallow water at a temperature of 75°–80°.

Stage 1. August 17th.—Length 138 mm.
,, 2. September 1st.
,, 3. September 15th.
,, 4. October 17th.
,, 5. October 24th.
,, 6. November 2nd.
,, 7. November 8th.
,, 8. November 14th.—Length 131 mm.

Total length of period—12 weeks.

*Specimen No. 2.*

Placed in shallow water at a temperature of 75°–80°.

Stage 1. August 17th.—Length 119 mm.
,, 2. September 1st.
,, 3. September 15th.
,, 4. November 9th.
,, 5. November 16th.
,, 6. November 21st.
,, 7. November 29th.
,, 8. December 4th.—Length 113 mm.

Total length of period—15 weeks

*Specimen No. 3.*

Placed in shallow water at a temperature of 55°–60°.

Stage 1. August 17th.—Length 124 mm.
„ 2. September 25th.
„ 3. October 10th.
„ 4. November 12th.
„ 5. November 20th.
„ 6. November 27th.
„ 7. December 5th.
„ 8. December 11th.—Length 118 mm.

Total length of period—16 weeks.

*Specimen No. 4.*

Placed in shallow water at a temperature of 55°–60°.

Stage 1. August 17th.—Length 105 mm.
„ 2. September 25th.
„ 3. October 10th.

Died January 22nd.—Length 105 mm.

*Specimen No. 5.*

Placed in foul water from which the oxygen was removed by the gradual addition of boiled water.

Stage 1. August 17th.—Length 136 mm.

Died January 4th.—Length 155 mm.

*Specimen No. 6.*

Placed in shallow water at a temperature of 75°–80°, and replaced in deep water as each successive stage was reached.

Stage 1. October 7th.—Length 134 mm.
„ 2. October 20th.

*Replaced in deep water.*

„ 1. October 28th.

*Replaced in shallow water.*

„ 2. November 15th.
„ 3. December 5th.

*Replaced in deep water.*

„ 2. December 20th.

*Replaced in shallow water.*

„ 3. January 10th.
„ 4. February 12th.

*Replaced in deep water.*

Stage 5. February 18th.
,, 6. February 25th.
,, 7. March 3rd.
,, 8. March 10th.—Length 129 mm.

*Specimen No. 7.*

Placed in shallow water at a temperature of 75°–80°.

Stage 1. October 7th.—Length 98 mm.
,, 2. October 31st.
,, 3. November 25th.

Died January 29th.—Length 98 mm.

*Specimen No. 8.*

Placed in shallow water at a temperature of 55°–60°.

Stage 1. October 7th.—Length 96 mm.
,, 2. October 20th.
,, 3. November 10th.
,, 4. January 3rd.
,, 5. January 10th.
,, 6. January 19th.
,, 7. January 27th.
,, 8. February 4th.—Length 91 mm.

Total length of period—16½ weeks.

*Specimen No. 9.*

Placed in shallow water at a temperature of 55°–60°, and replaced in deep water as each successive stage was reached.

Stage 1. October 7th.—Length 117 mm.
,, 2. October 25th.

*Replaced in deep water.*

,, 1. November 5th.

*Replaced in shallow water.*

,, 2. November 20th.
,, 3. December 26th.

*Replaced in deep water.*

,, 2. January 10th.

*Replaced in shallow water.*

,, 3. January 25th.
,, 4. February 28th.

*Replaced in deep water.*

Stage 5. March 6th.
„ 6. March 13th.
„ 7. March 19th.
„ 8. March 27th.—Length 113 mm.

*Specimen No. 10.*

Placed in shallow water at a temperature of 55°–60°.

Stage 1. October 7th.—Length 105 mm.
„ 2. October 31st.
„ 3. November 30th.

Died March 24th.—Length 105 mm.

*Specimen No. 11.*

Placed in foul water from which the oxygen was removed by the gradual addition of boiled water.

Stage 1. October 7th.—Length 112 mm.
Died March 22nd. „ 120 mm.

The results of these experiments show, I think, in the first place that, in accordance with Mlle. de Chauvin's observations, and contrary to those of Dr. Powers, the Mexican Axolotl will, with perhaps a few exceptions, transform into the Amblystome stage if placed, when about six months old, under conditions which force it to make frequent use of its lungs; secondly, that starvation, irregular feeding, and temperature have no influence on the metamorphosis; thirdly that, as no change occurs when the Axolotl is placed in poorly oxygenated water, owing to the fact that it will not under the circumstances rise to the surface and make use of its lungs, the quantity of oxygen in the lakes of Mexico can have little bearing on the explanation of the phenomenon of neoteny; and lastly, that there is a critical stage in the metamorphosis.

*References.*

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2. A. Weismann.—Zeitschr. wiss. Zool. xxv. (1875).
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